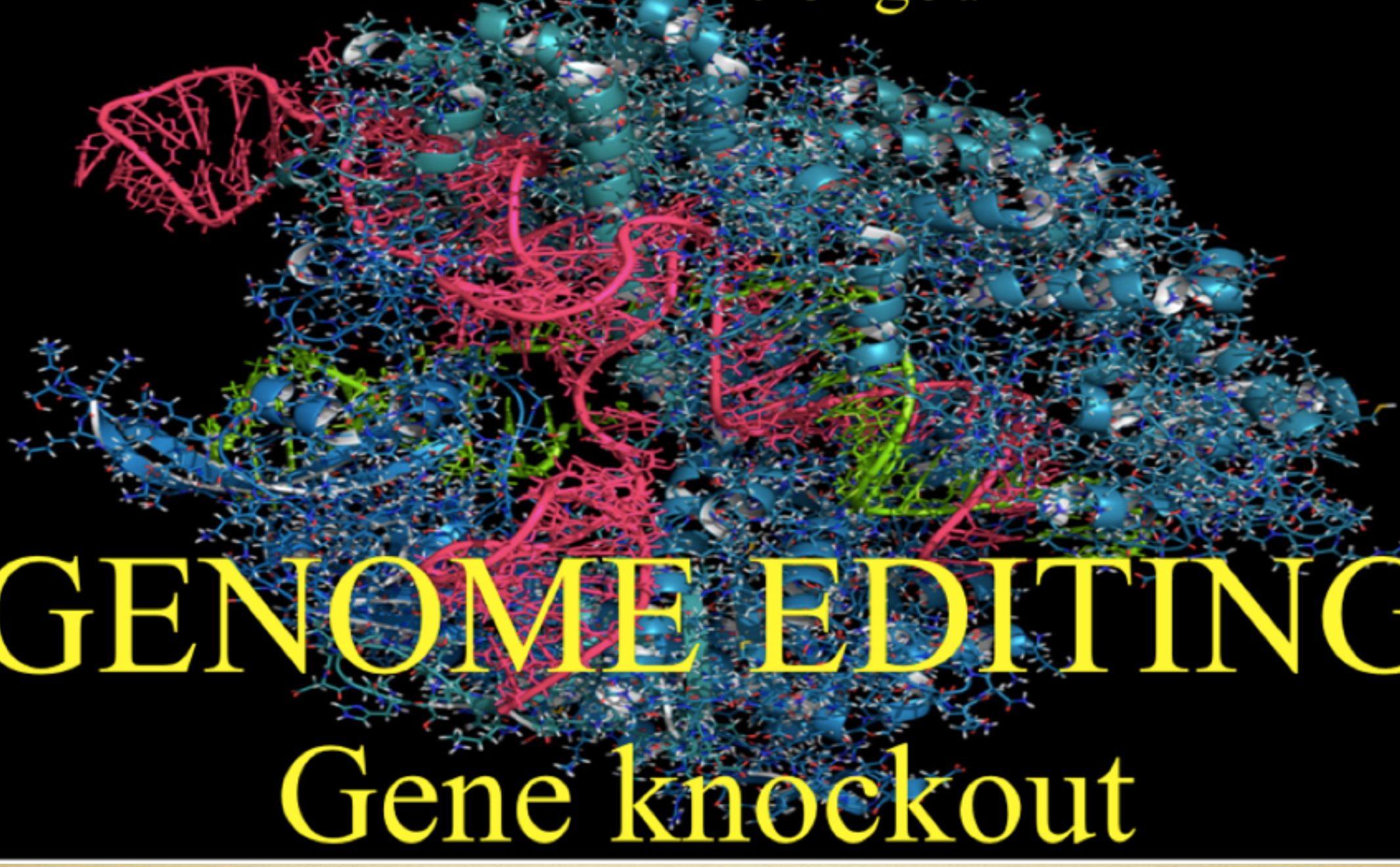
in the name of god



GENOME EDITING

Gene knockout

presented by Maryam Barghbani
Under supervision of Dr Ahmadpour Yazdi

content

- History
- Introduction
- Application
- Conclusion

History (8)

- in the recent decay
- major advances have been made in the development of Genome Editing (GE) methods
- the first successful editing of a mammalian genome was performed on mice in the 1980

Introduction (8)

- Genome editing (is a technique for)
- Deleting
- Replacing
- Adding

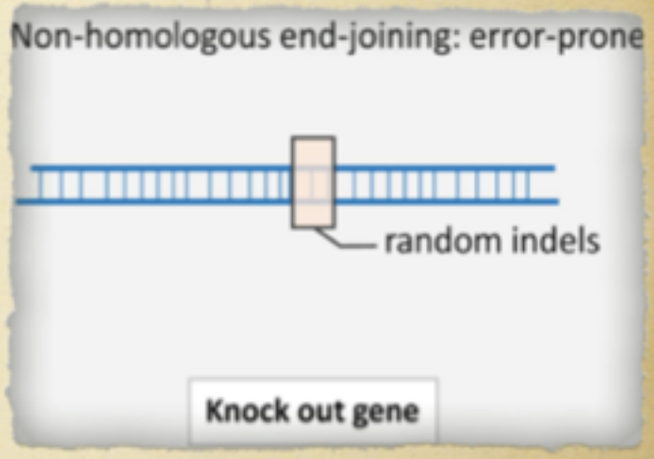
Genome Editing pathway (1)

DSB(Double Strand Break)

NHEJ (Non_ homologous end joining : error _ prone) for Indel

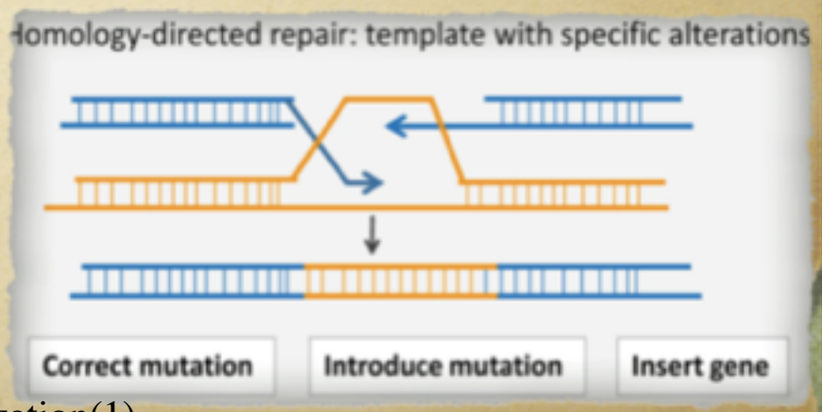
Deletion
Insertion

Knock out gene



HDR (Homology_direct repair:template with specific a for

gene Correction
modification
Gene adding
mutation



High-Content Analysis of CRISPR-Cas9 Gene-Edited Human Embryonic Stem Cells
International regulatory landscape and integration of corrective genome editing into in vitro fertilization(1)

Technology GE

1. microRNA & siRNA
2. Antisense Oligonucleotides (ASOs)
3. Nuclease

ZFN
TALEN
CRISPER-Cas9

The more commonly known Nucleases are (8)

- ☑ Zinc Finger Nuclease (ZFN)
- ☑ Transcription Activator- Like Effector Nucleases (TALEN)
- ☑ Clustered Regulatory Interspersed Short Palindromic Repeats (CRISPR)
- ☑ These methods differ in the way they recognize a target location.

Application

- **Disease Modeling**
- **Animal Models**
- Target Disease
- Gene Therapy
- iPSCs (Induced Pluripotent Stem Cells)
- cancer models

Timeline of milestones (2)

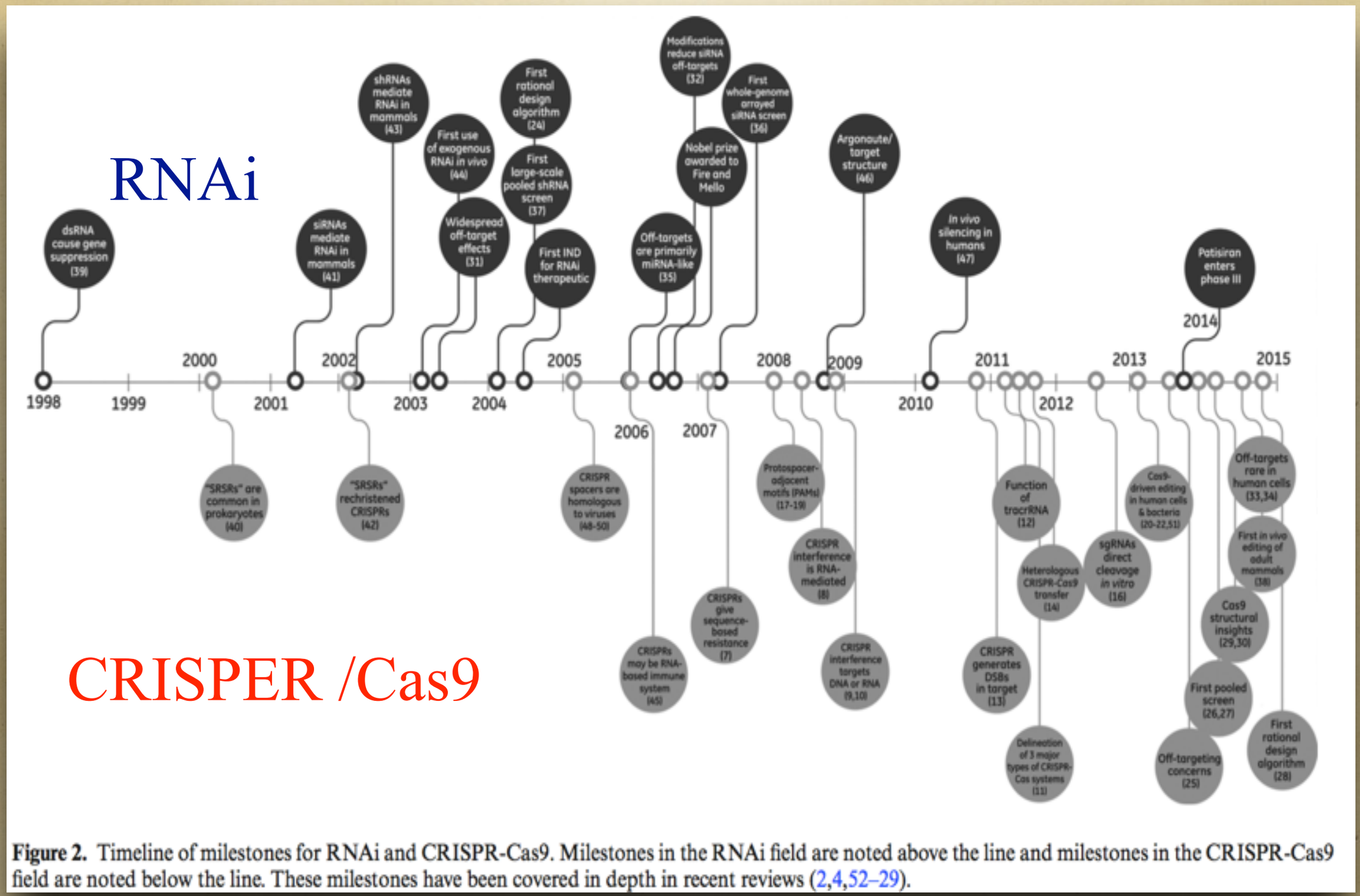


Figure 2. Timeline of milestones for RNAi and CRISPR-Cas9. Milestones in the RNAi field are noted above the line and milestones in the CRISPR-Cas9 field are noted below the line. These milestones have been covered in depth in recent reviews (2,4,52–29).

Timeline of GE

- 2011 = ZFN pig and cattle
- 2012 =TALEN = pig
- 2013 =ZFN and TALEN = pig
- 2014 = CRISPR/Cas9 =pig and sheep
- 2015 = TALEN = cattle and goat

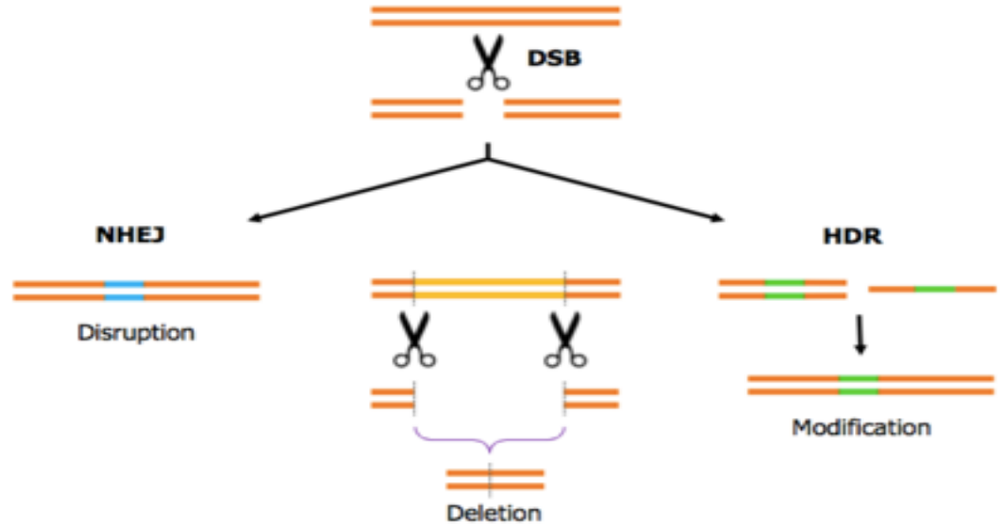


Fig. 2 The utility of double strand breaks generated by genome editors. A cartoon depiction of the double strand break (DSB) repair mechanisms. Non homologous end joining (NHEJ) is an error prone process that re-joins the end of the DSB, often resulting in small insertions/deletions (*blue*) and subsequent gene disruption. Homology dependent repair is a faithful process that uses a homologous template to repair the DSB. Providing a repair template, either as a single stranded oligonucleotide or double stranded DNA, allows specific modifications (*green*) to be introduced to the genome. Creation of simultaneous DSBs flanking a region of the genome can result in deletion of the intervening sequence (*yellow*) and repair of the DSBs by either NHEJ or HDR. (Color figure online)

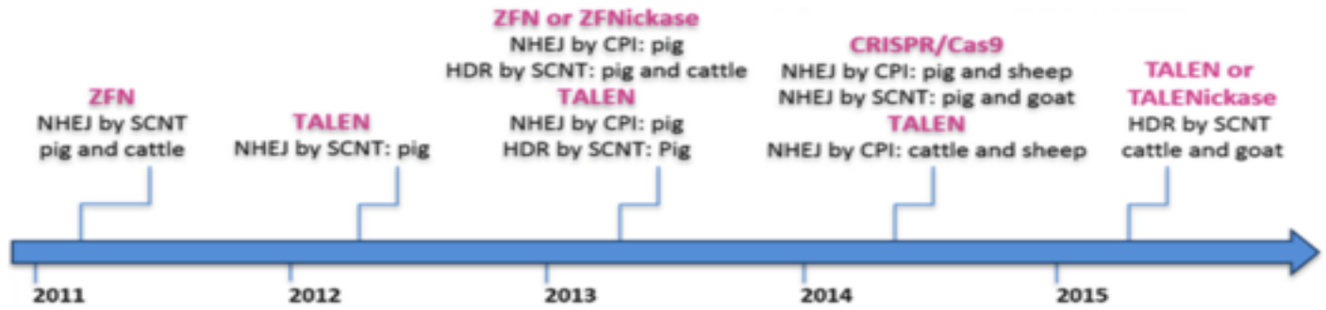
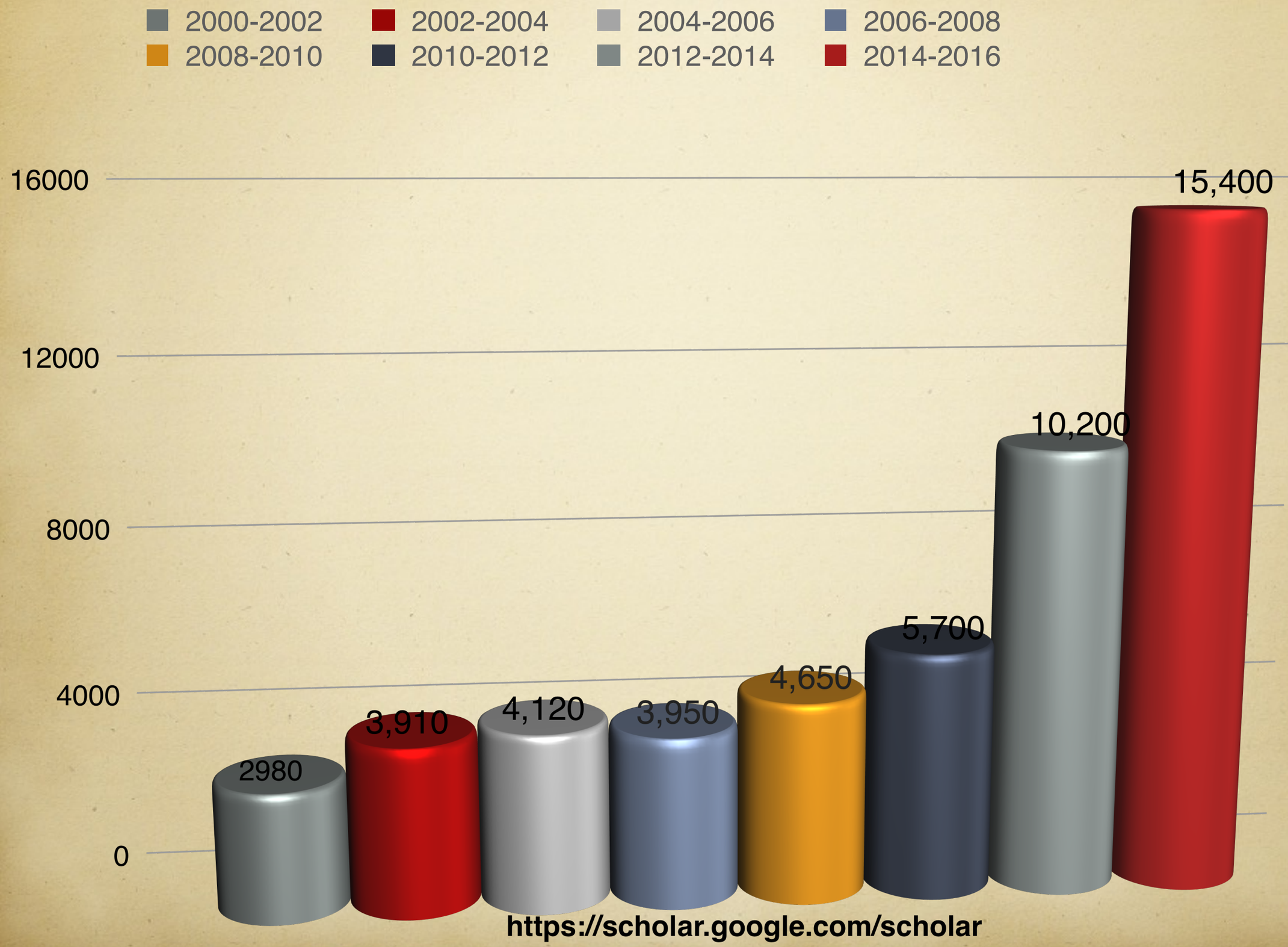


Fig. 3 A Timeline of genome edited livestock over the past 5 years highlighting specific milestones

Gene targeting, genome editing: from Dolly to editors

CRISPER/Cas9



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Genome editing pathway(1)

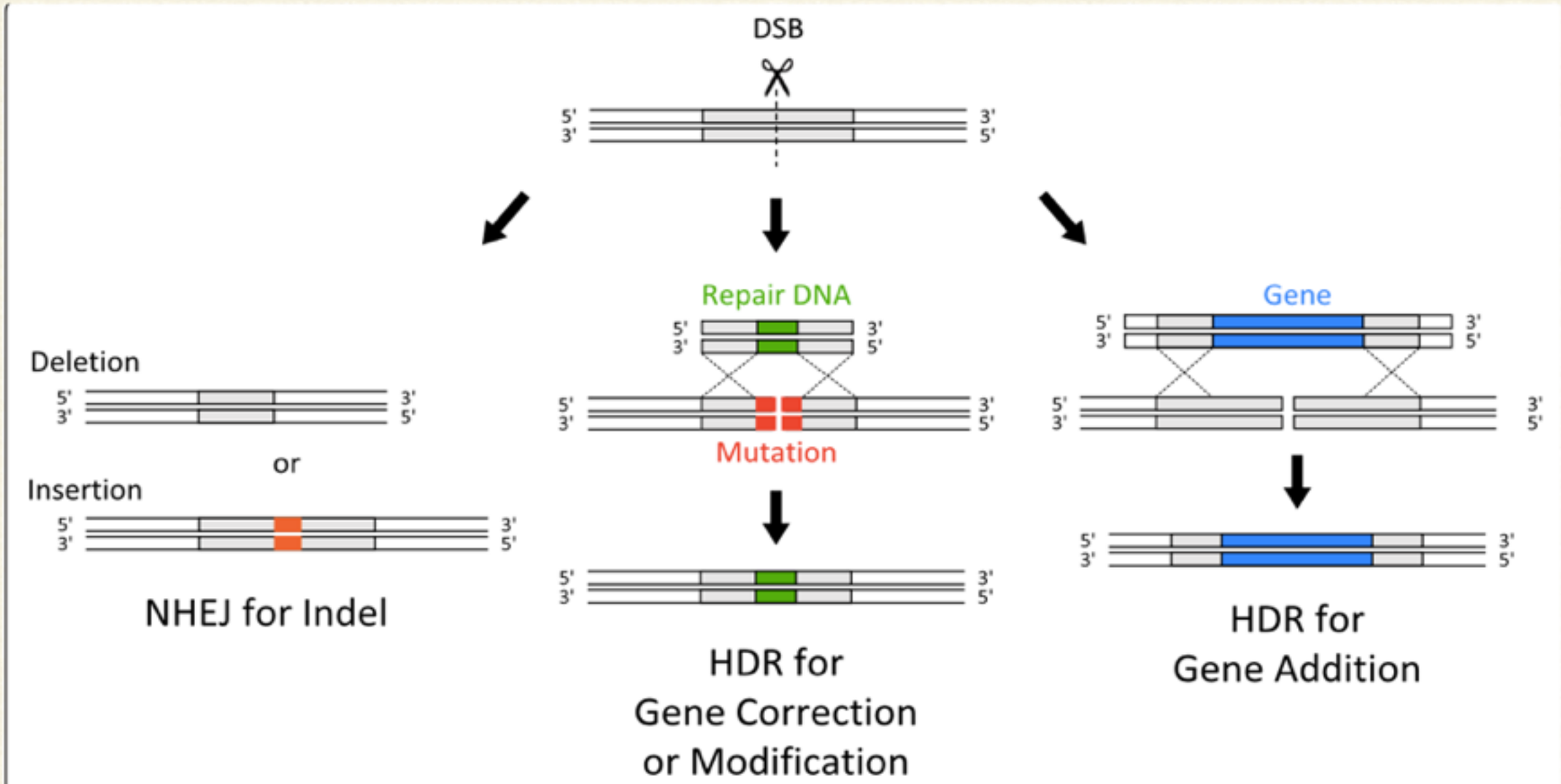


Figure 1 Engineered nuclease-induced genome editing pathways. Double-stranded breaks (DSBs) are induced at a targeted sequence by a microorganism-originated, engineered nuclease. Non-homologous end-joining (NHEJ) is a DSB repair pathway that ligates or joins two broken ends together, resulting in the introduction of small insertions or deletions (indels) at the site of the DSB. Homology-directed repair (HDR) is a DNA template-dependent pathway for DSB repair, using a homology-containing donor template along with a site-specific nuclease, enabling the insertion of single or multiple transgenes (gene addition) in addition to single-nucleotide substitutions in which an amino acid substitution of a protein occurs (gene modification), or a mutation is completely repaired in the resultant organism genome (gene correction).

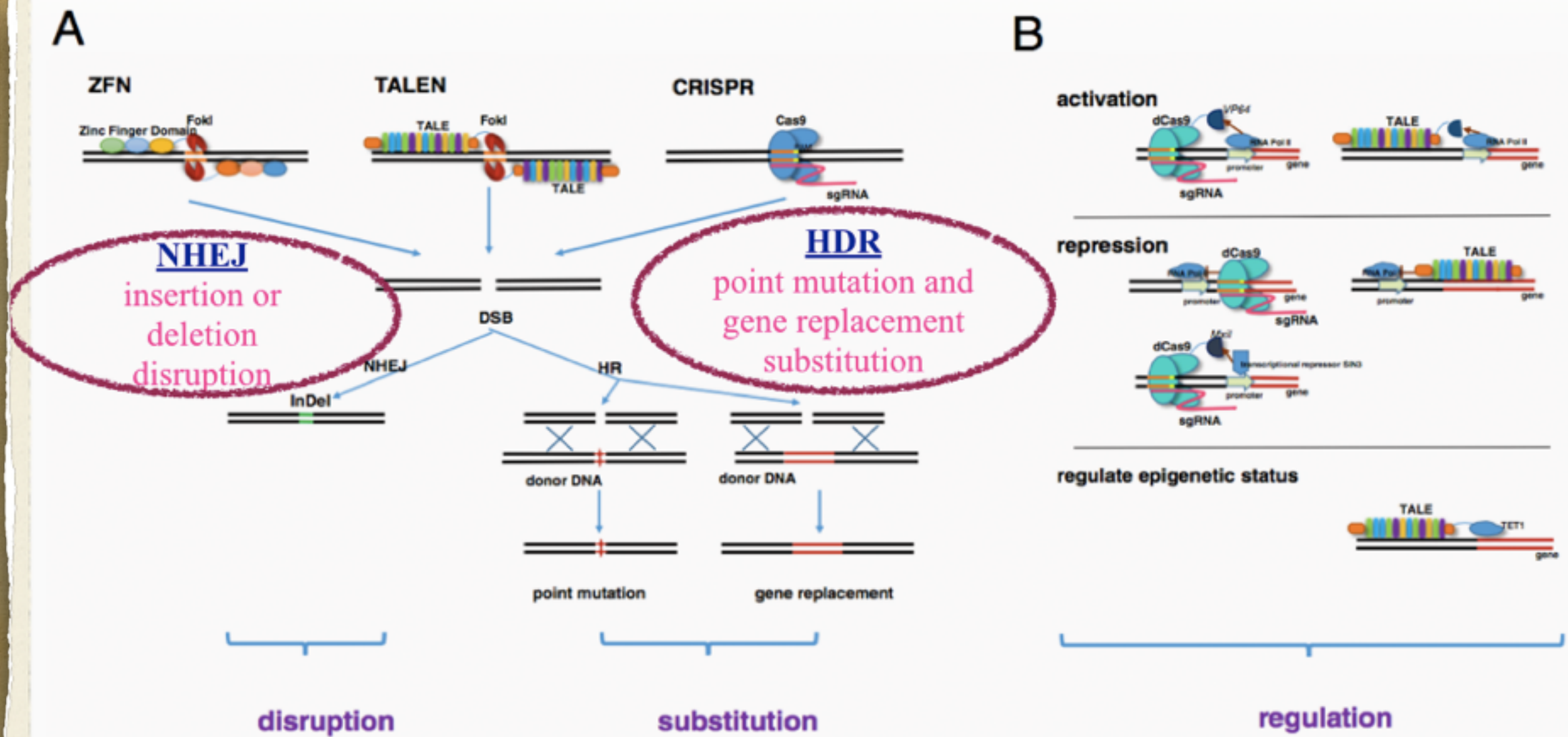


Figure 1 Mechanism of ZFN, TALEN and CRISPR/Cas9. A. ZFN, TALEN and CRISPR/Cas9 achieve precise and efficient genome modification by inducing targeted DNA DSBs, which would be corrected by NHEJ and HR repair mechanisms. NHEJ-mediated repair leads to the introduction of variable length insertion or deletion. HR-mediated repair could lead to point mutation and gene replacement, in the present of donor DNA. **B**. TALEs and Cas9 protein fused with effector proteins such as VP64, Mxi1 could regulate expressions of endogenous genes. Additionally, TALEs fused with histone-deacetylating epigenetic effectors could regulate epigenetics status of endogenous genes. CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; dCas9, inactive Cas9 protein; DSB, Double Strand Breaks; NHEJ, Error-prone Nonhomologous End Joining; HR, Homologous Recombination; InDel, Insertion and Deletion; PAM, Protospacer Adjacent Motif; RNA Pol II, RNA Polymerase II; sgRNA, single guide RNA; TALE, Transcription Activator-Like Effector; TALEN, Transcription Activator-Like Effector Nuclease; ZFN, Zinc Finger Nuclease.

Advances in genome editing technology and its promising application in evolutionary and ecological studies (5)

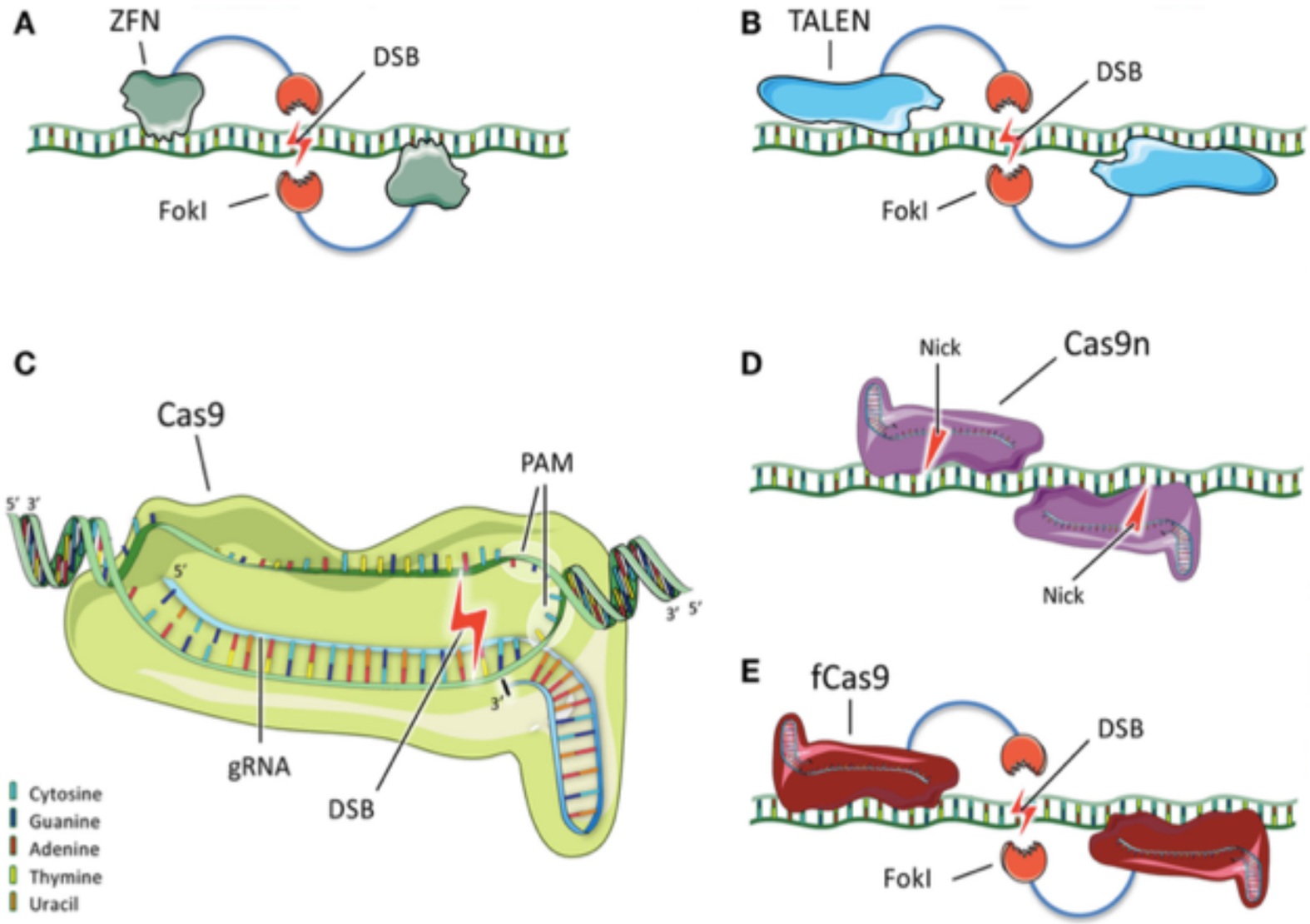


FIGURE 2 | Schematic representation of ZFNs, TALENs, and CRISPR/Cas9. **(A)** Two ZFN dimers bind DNA and position their *Fok*I nuclease domains such that they dimerize and generate a double-stranded break (DSB) between the binding sites. **(B)** TALENs, like ZFNs, bind DNA and generate a DSB upon dimerization of their *Fok*I domains. **(C)** In the most commonly used CRISPR/Cas9 system, Cas9 forms a complex with a gRNA that recognizes and hybridizes a 20-bp protospacer in the genome. Cas9 binds the adjacent PAM sequence and introduces a DSB 3 bp upstream of the PAM sequence. **(D)** Cas9 nickases (Cas9n) are mutant variants that bind to flanking DNA sequences and generate single-strand nicks instead of DSBs. Two nicks are the equivalent of a DSB. **(E)** Another variant consists of catalytically inactive Cas9 (fCas9) fused to a *Fok*I nuclease domain. When two *Fok*I nucleases dimerize because the Cas9 proteins bind to flanking DNA sequences, a DSB is introduced between the binding sites.

High-Content Analysis of CRISPR-Cas9 Gene-Edited Human Embryonic Stem Cells (3)

pathway of Nuclease

1 = microRNA & siRNA

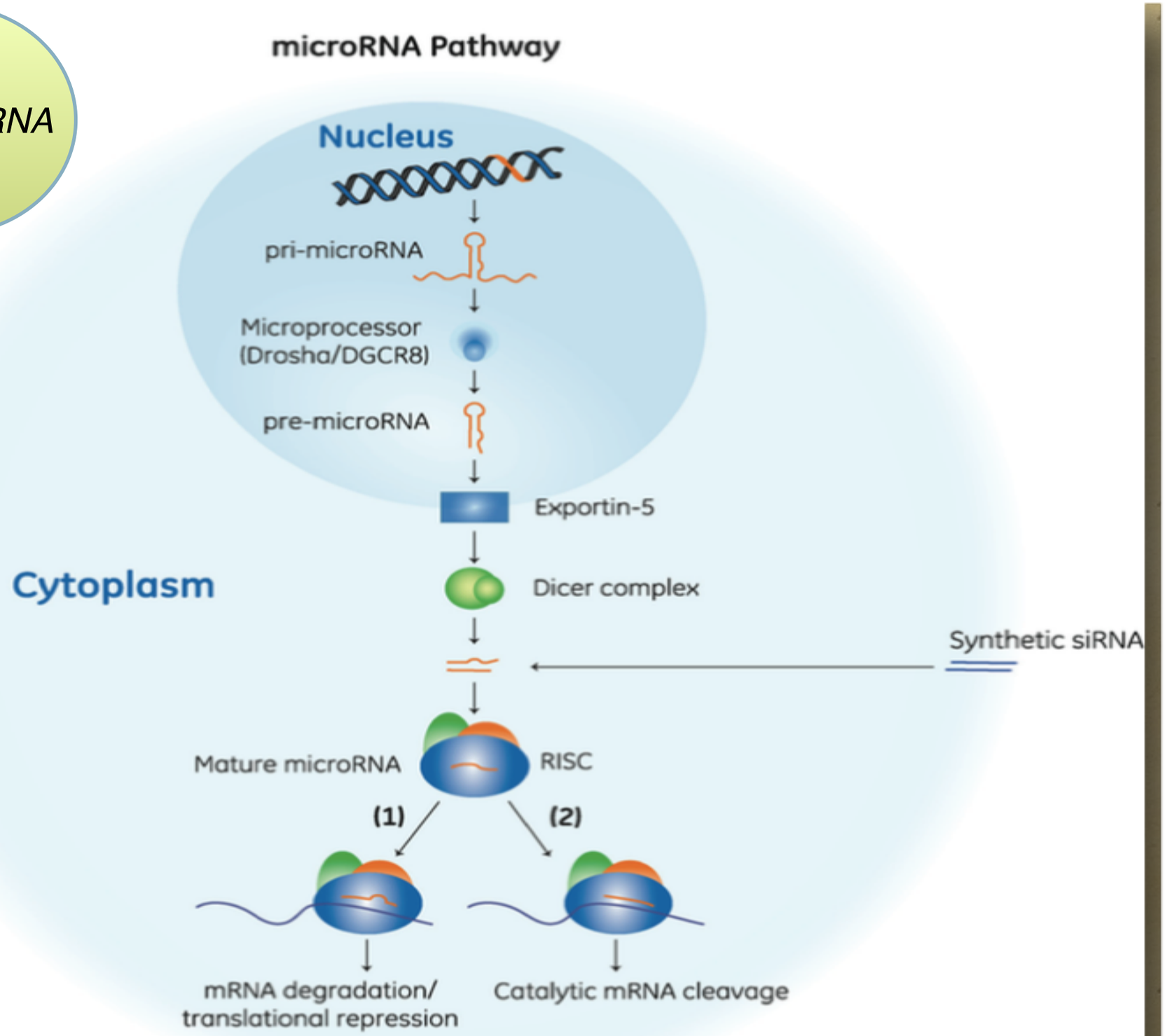
2 = ASOs

3 = ZFN

4 = TALEN

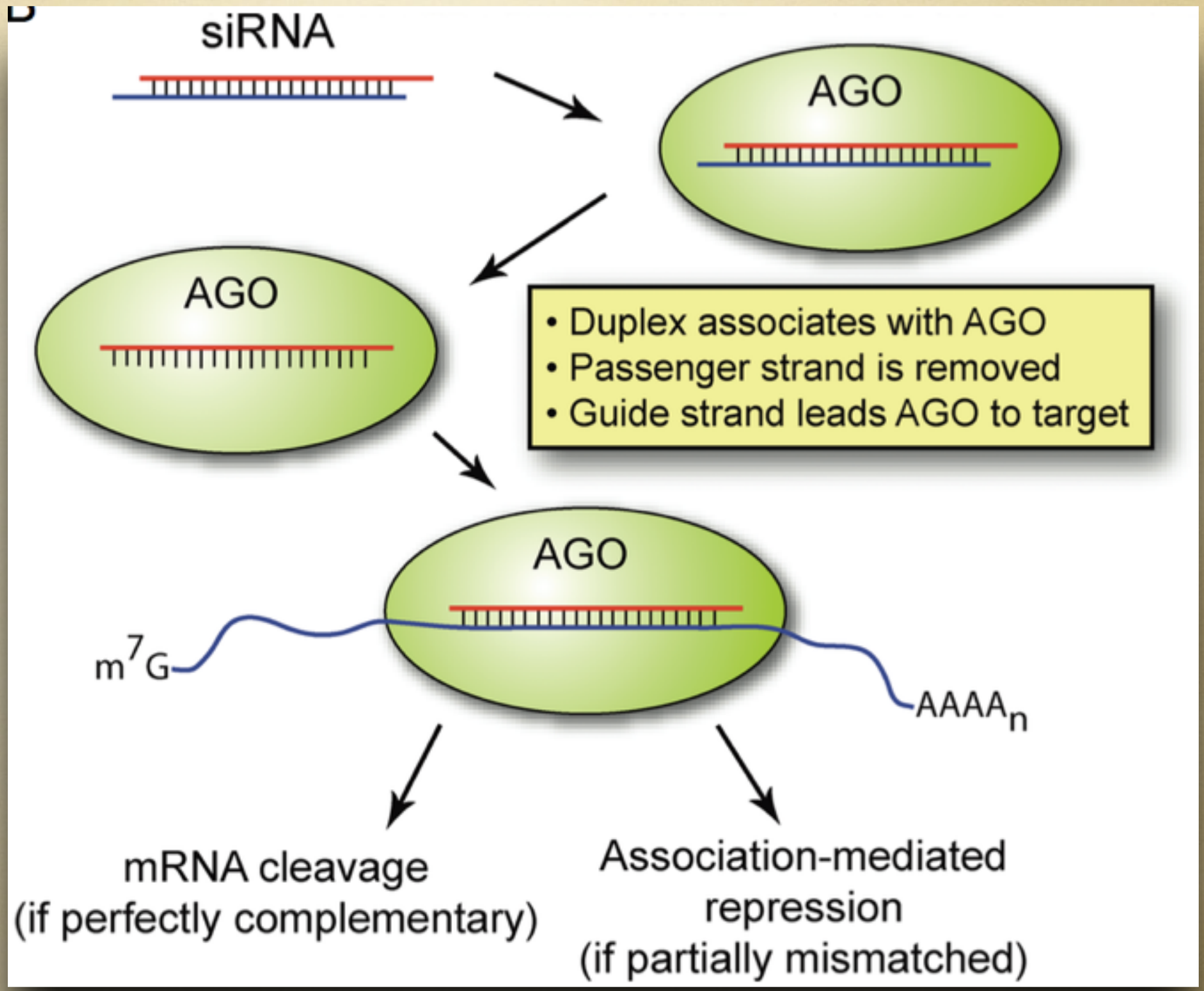
5 = CRISPER/Cas 9

1= *microRNA*



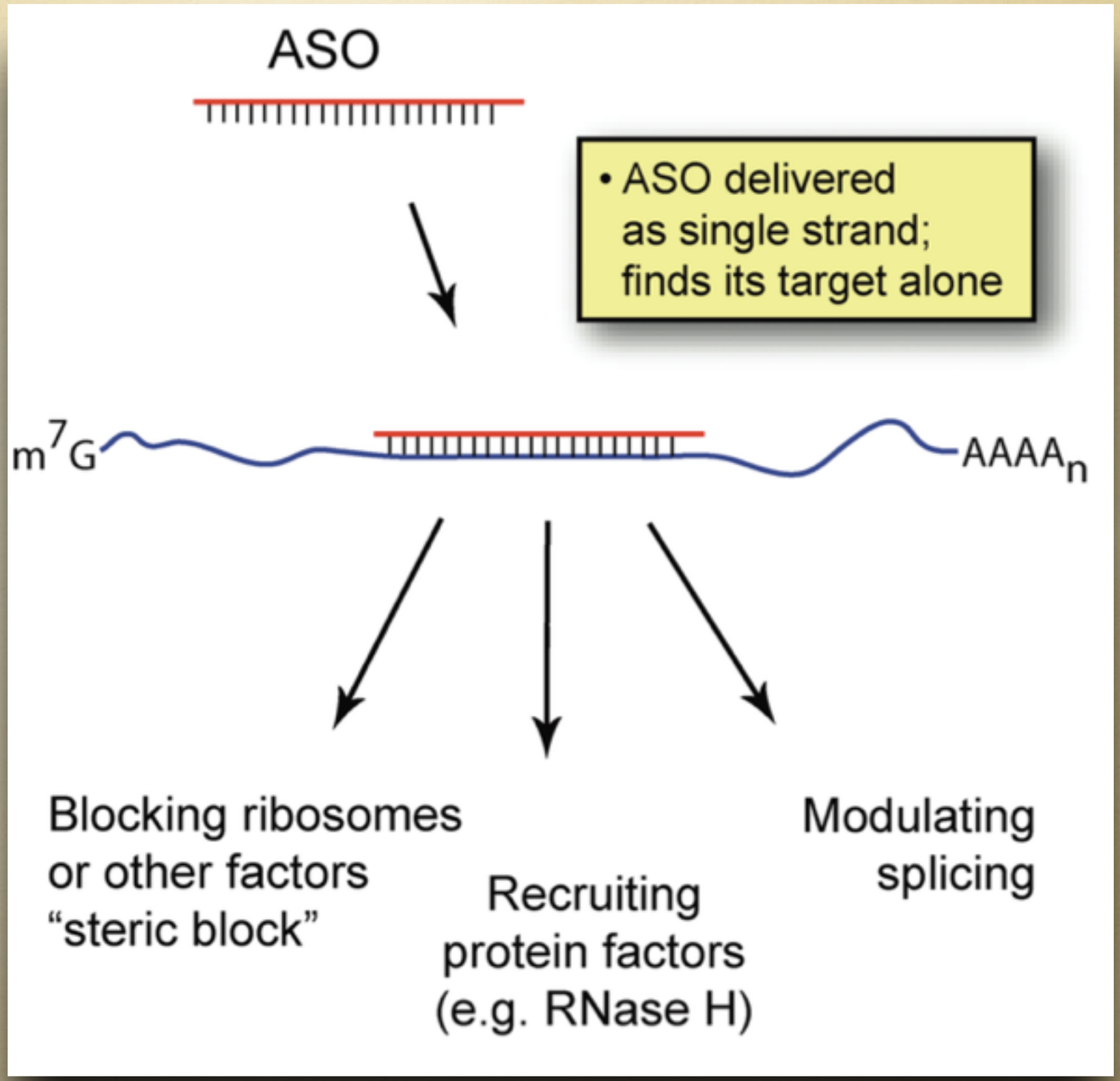
Advances in CRISPR-Cas9 genome engineering/ lessons learnd from RNA interferencee (2)

1= *siRNA*



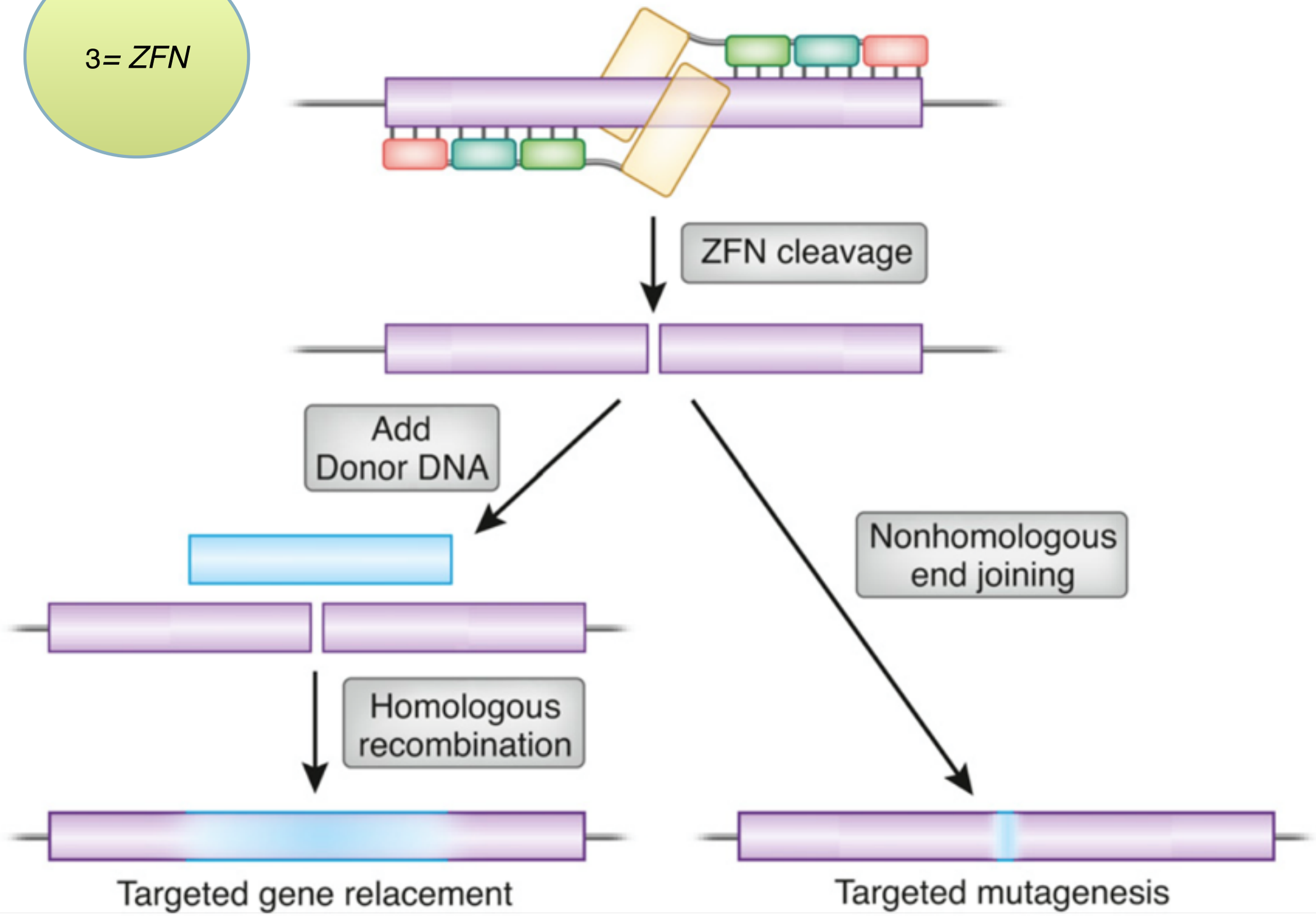
Silencing disease genes in the laboratory and the clinic (22)

3 = ASO



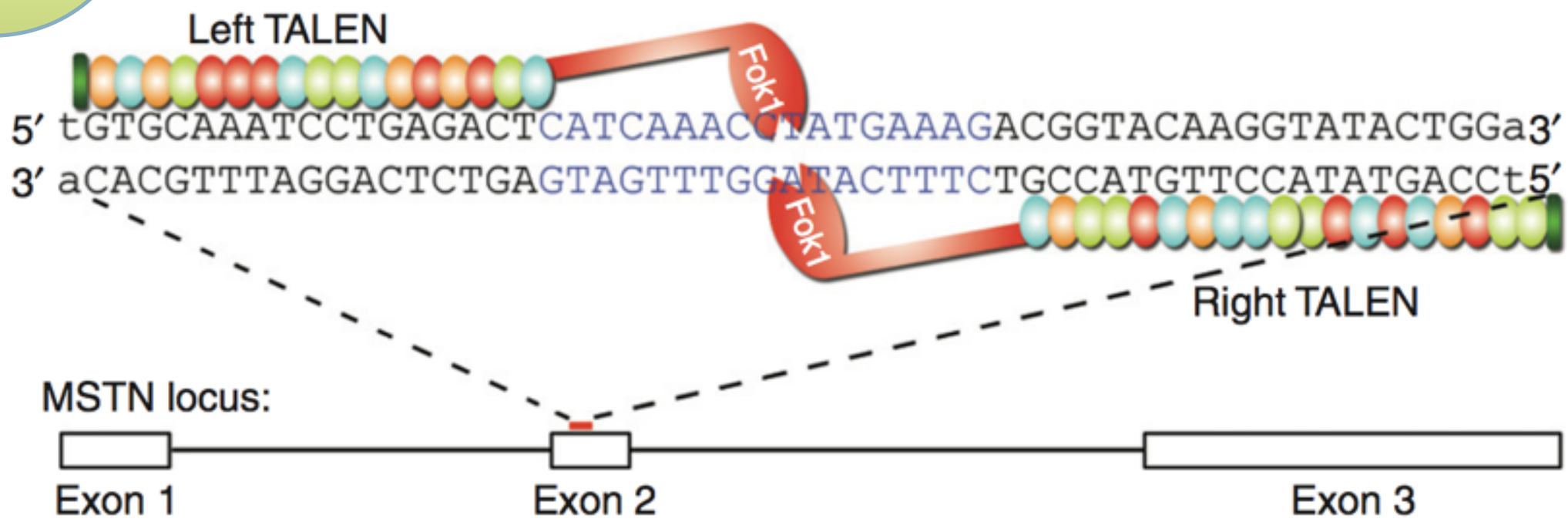
Silencing disease genes in the laboratory and the clinic (22)

3= *ZFN*



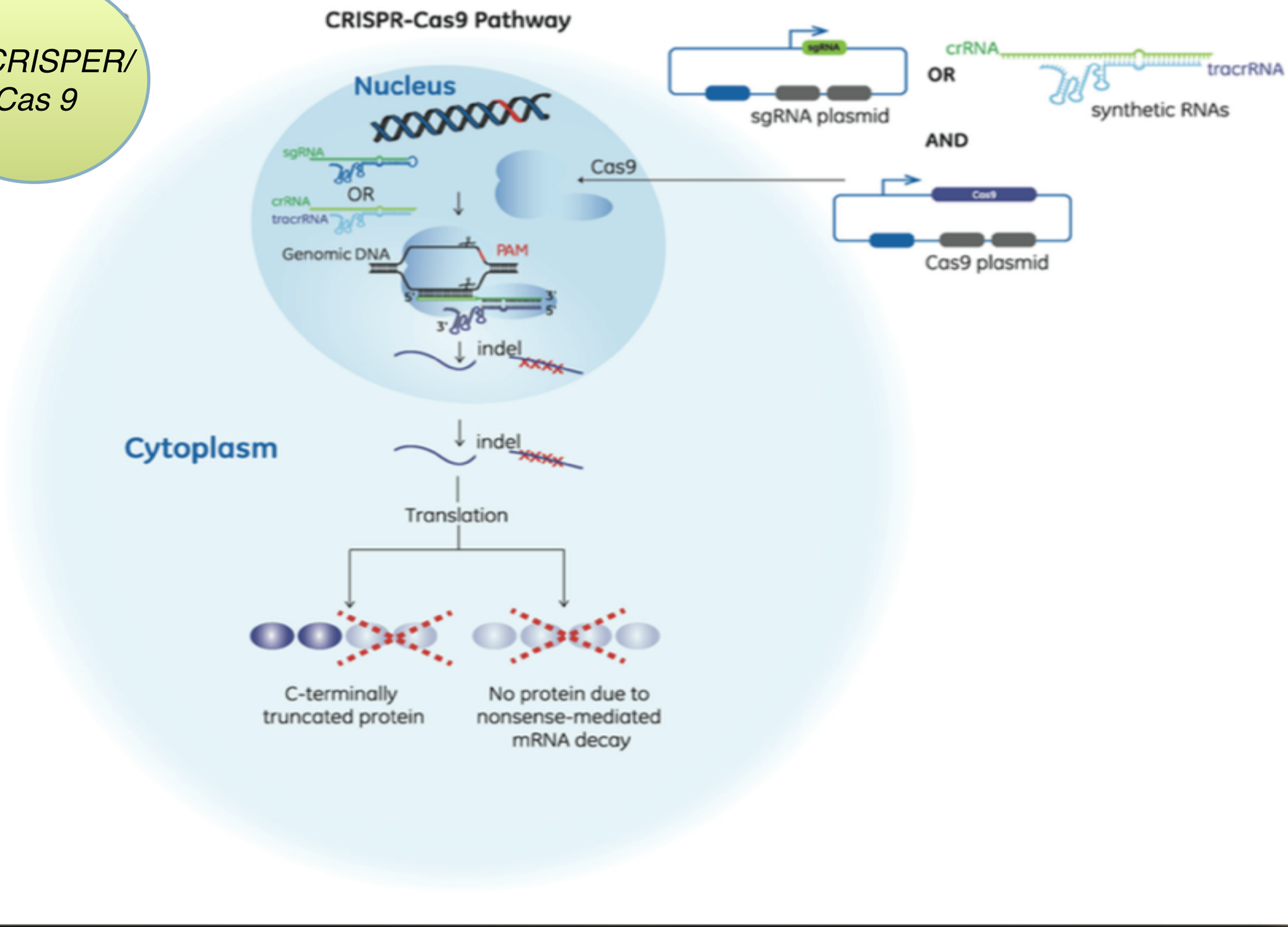
Genome Engineering With Zinc-Finger Nucleases (21)

4 =TALEN



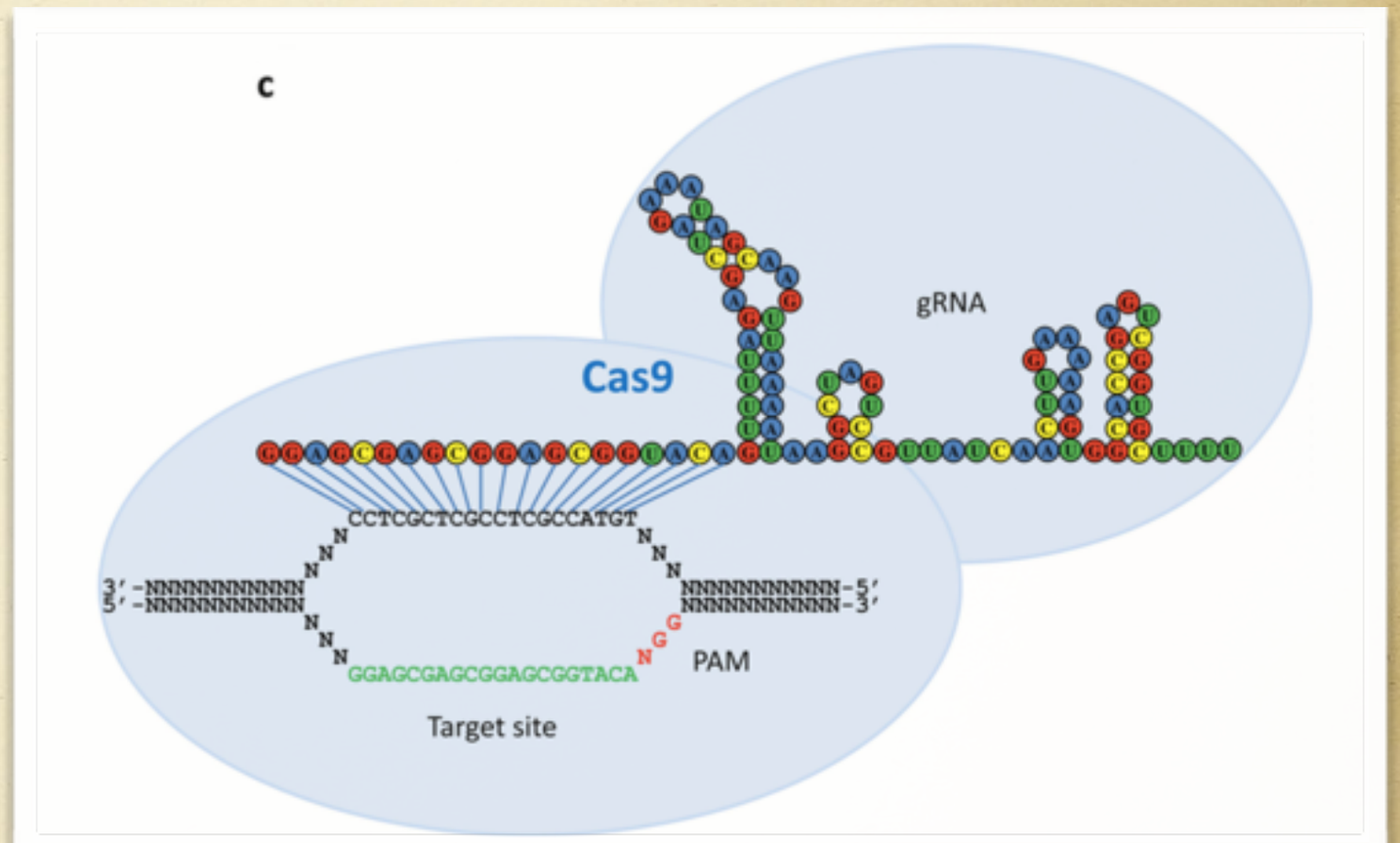
Targeted Myostatin Gene Editing in Multiple Mammalian Species Directed by a Single Pair of TALE Nucleases(18)

5= CRISPER/ Cas 9



structure of CRISPER Cas9(7)

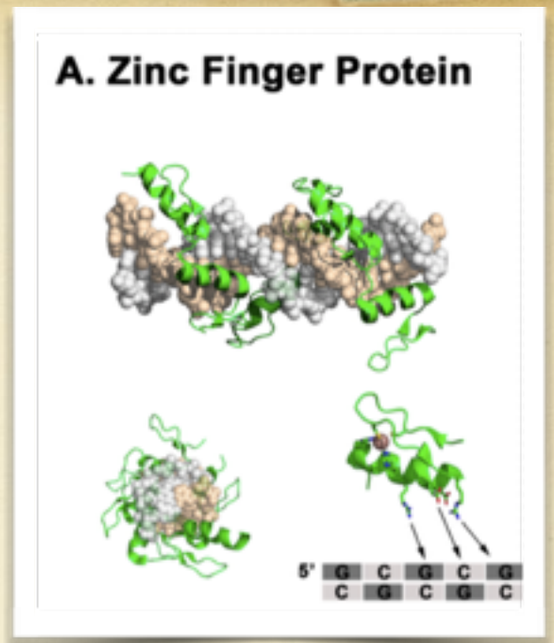
- Cas9 protein
- guide RNA
- tracer RNA
- **PAM**(proto spacer adjacent motif) NGG

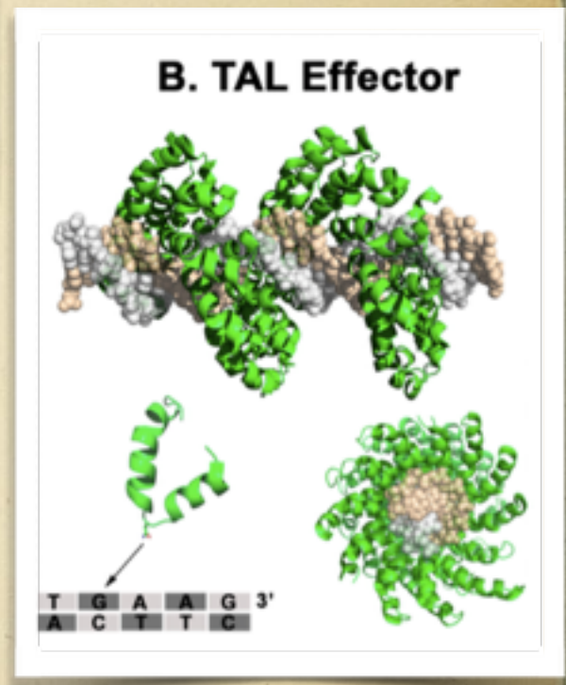


Efficient In Vivo Genome Editing Using RNA-Guided Nuclease

comparison

between different genome editing technologies





ZFN

TALEN

CRISPER

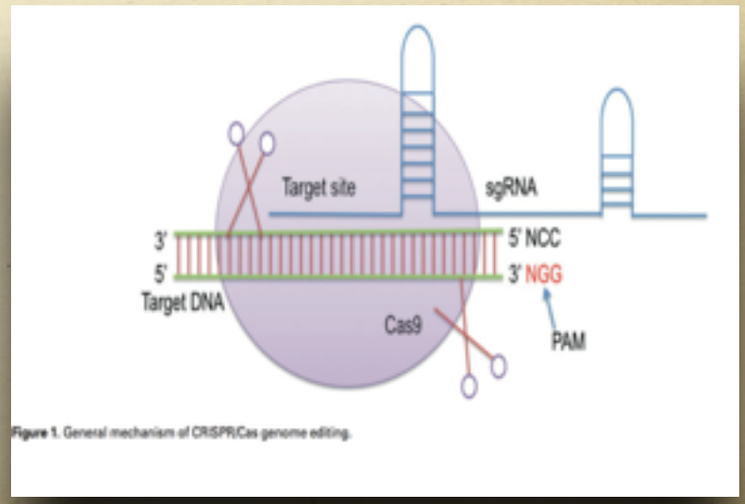


Figure 1. General mechanism of CRISPR/Cas genome editing.

Feature	ZFN	TALEN	CRISPR/Cas9
Efficiency	12%	76%	81%
Off -target effect	more potential off-target effect than TALENs	less observed	more potential off target than other
methylation sensitive	sensitive	sensitive	Not sensitive
target DNA recognition	Zinc fingers (ZFs)	Repeat variable diresidues (RVDs)	crRNA or sgRND
Minimum number of DNA base be recognized	Triple	Single	Single
Size of recognized DNA sequences	9-18 bp	(8-13 bp)*2	20 bp +NGG
Cytotoxicity	High	Low	Low
advantage (CRISPER-Cas9 than (other	High efficiency		Very high efficiency and rapid construction and easy delivery
Disadvantage	More difficult to assemble than CRISPR and remaining possibility of off target cleavage		Off-target cleavage possibly more frequent than ZFN and TALEN
Target range	Limited by range and context-dependence of ZF modules		Limited by PAM sequence (potentially unlimited)
Success rate of nuclease design	Low	High	High
Made of action	DNA breaked by protein-DNA recognition	DNA break targeted by protein-DNA recognition	DNA break targeted by RNA-DNA recognition

(2)

comparison of approaches for gene knock down & gene knock out (14)

Feature	RNAi	CRISPR/Cas9
Mode of action	Knock down gene at mRNA or non coding RNA level	Modifies gene (via knock out / knock in)at genomic DNA level
	cytoplasm	nucleus
Duration of effect	Transient (siRNA)	permanent heritable change genome
Efficiency	Typically induces >75% knock down	Typically induces 10-40% editing per allele
Design of functional components	Gene employ reagent targeted all along transcript	Can employ only reagent with targets adjacent to PAM and (for gene knockout) in critical exon
Target substrate	RNA	DNA
Gene expression modulation	knockdown	knockdown , over expression and knockout
library creation from genomic DNA/DNA	Yes	No
Accessory proteins	RISC	Cas9

Table 1 | **Comparison of approaches for gene knockdown or knockout** (6)

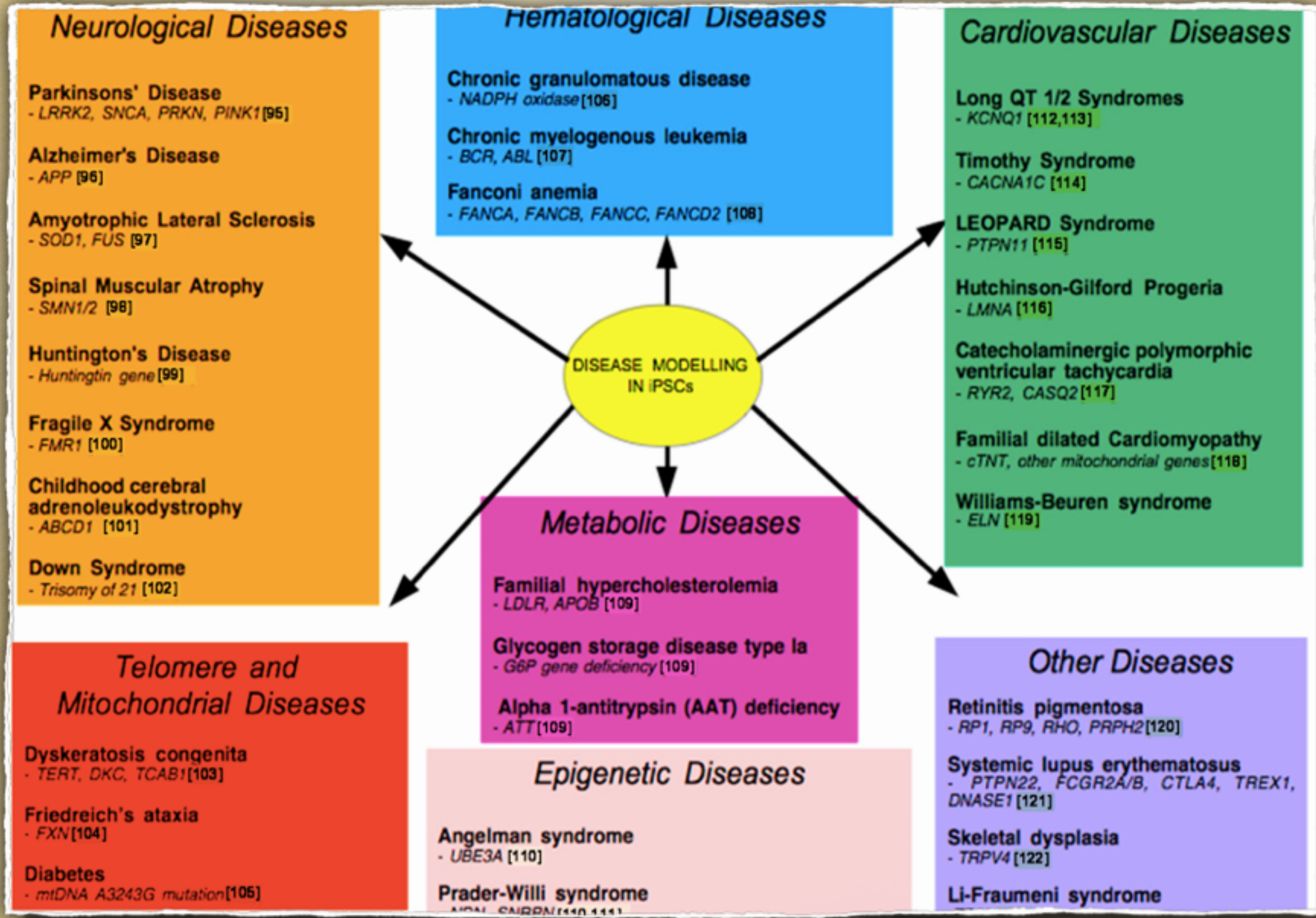
	Approach				
	RNAi	**ASO**	**ZFN**	**TALEN**	**CRISPR–Cas**
Molecular target	RNA	RNA	DNA	DNA	DNA
Result of targeting	Reversible knockdown	Reversible knockdown	Irreversible knockout	Irreversible knockout	Irreversible knockout
Ease of generating target specificity	Easy: simple oligo synthesis and cloning steps and limited chemical modifications to enhance RNA degradation	Easy: simple oligo synthesis and cloning steps; often chemical modification is required to enhance RNA binding and ASO stability	Difficult: substantial cloning and protein engineering required	Moderate: substantial cloning steps required	Easy; simple oligo synthesis and cloning steps
Off-target activity	High	High	Moderate	Low	Low
Ease of multi-plexing	High	High	Low	Moderate	High
Transcriptional and epigenetic control	Direct control not possible	Direct control not possible; TSOs can interfere with protein translation	DNA-binding ZF domains can be fused to new functional domains	DNA binding domains can be fused to new functional domains	Enzymatically inactive dCas9 can be fused to new functional domains
Ease of delivery into the mammalian CNS	High: delivered by nanoparticles, bioconjugates, cell-penetrating peptides or viral vectors	High: delivered by nanoparticles, bioconjugates, cell-penetrating peptides or viral vectors	Moderate: delivered by viral vectors	Moderate: delivered by viral vectors but large size makes packaging into viral vectors challenging	Moderate: delivered by electroporation, PEI-mediated transfection, nanoparticles and viral vectors
Ease of generating large-scale libraries	High: simple oligo synthesis and cloning required	High: simple oligo synthesis and cloning required	Low: complex protein engineering required for each gene	Moderate: technically challenging cloning steps	High: simple oligo synthesis and cloning required
Costs	Low	Low	High	Moderate	Low

ASO, DNA antisense oligonucleotide; Cas, CRISPR-associated protein; CRISPR, clustered regularly interspaced short palindromic repeat; dCas9, dead Cas9; PEI, polyethylenimine; RNAi, RNA interference; TALEN, transcription activator-like effector nuclease; TSOs, translation-suppressing oligonucleotides; ZF, zinc-finger; ZFN, ZF nuclease.

Application

- **Disease Modeling**
- **Animal Models**
- Target Disease
- Gene Therapy
- iPSCs (Induced Pluripotent Stem Cells)
- cancer models

Induced Pluripotency and Gene Editing in Disease Modeling(13)



Induced Pluripotency and Gene Editing in Disease Modelling: Perspectives and Challenges

- Neurological Diseases (13)
- Hematological Diseases
- Telomere & Mitochondrial Diseases
- Metabolic Diseases
- Epigenetic Diseases
- Cardiovascular Diseases
- other Diseases

Animal Models (19)

- rabbits
- (with IL2 , RAG1 , RAG2 knockout)
- hamsters
- (with STAT knock out)

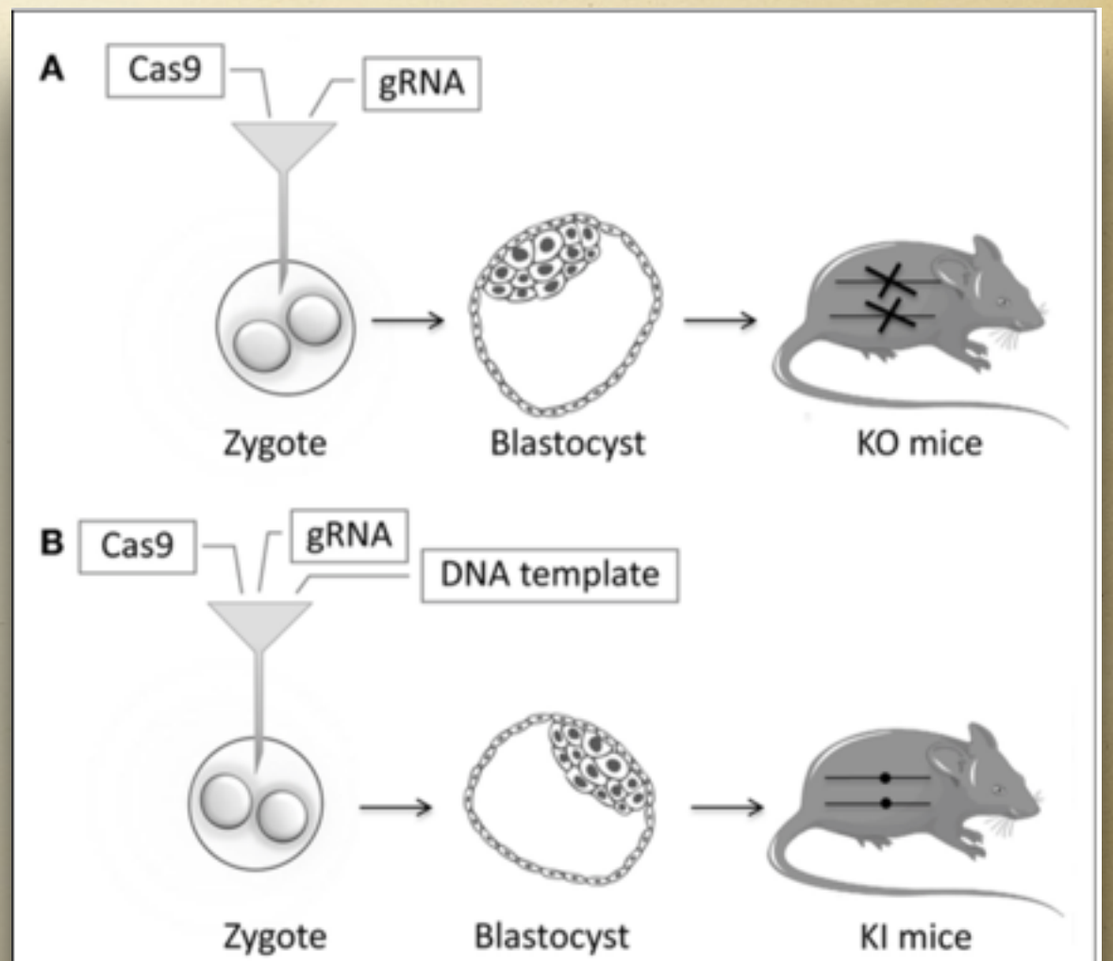


FIGURE 4 | Schematic representation of zygote injection with CRISPR/Cas9. **(A)** Injection of gRNA and Cas9 will lead to indels that can lead to a frameshift and an early stop codon thereby creating Knockout (KO) mice. **(B)** Addition of a highly homologous DNA template containing a specific mutation will result in Knock-in (KI) mice, through the process of homology-directed repair (HDR). Reagents are injected in the cytoplasm of the zygote. Alternatively, these can be injected in the pronucleus of the zygote, but cytoplasmic microinjection is simpler and less toxic.

- Novel genome-editing tools to model and correct primary immunodeficiencies

CRISPER -Cas9 for target disease (2)

Table 2. Summary of publications demonstrating use of CRISPR-Cas9 for targeting disease

Disease	Summary
Cataracts	Rescue of a dominant mutation in the Crygc gene that causes cataracts (108).
Cystic fibrosis	Correction of the CFTR locus by homologous recombination in cultured intestinal stem cells from patients with cystic fibrosis (109).
β-thalassemia	Correction of the human hemoglobin beta (HBB) gene in induced pluripotent stem cells from β-thalassemia patients using CRISPR-Cas9 and the piggyback transposon (106).
HPV-associated cervical cancer	Targeting of promoters of human papillomavirus oncogenes; inhibited tumorigenesis (110).
Hereditary tyrosinemia type I	Correction of the Fah mutation in hepatocytes of a mouse model of hereditary tyrosinemia (107).
HIV	Generation of homozygous CCR5 deletion mutations in iPSCs; proposed approach toward a functional cure of HIV-1 infection (111). Targeting of LTR sequences in the HIV-1 genome; inactivated viral gene expression and replication in latently infected cells and prevented new HIV-1 infection (112).
Malaria	High (50–100%) gene disruption of the *Plasmodium falciporum* genome. Potential to generate transgenic parasites to prevent malaria (113).
Duchenne Muscular Dystrophy (DMD)	2–100% correction of the DMD mutation in the dystrophin gene in the germ line of a mouse model of DMD (114).
Herpesviridae infection	Targeting of genomes of latent herpesviridae viral infections; suggests use as an antiviral treatment in human cells (115).

Advances in CRISPR-Cas9 genome engineering: lessons learned from RNA interference

(gene therapy & disease modeling)(17)

Genome-editing tools for stem cell biology
EA Vasileva *et al*

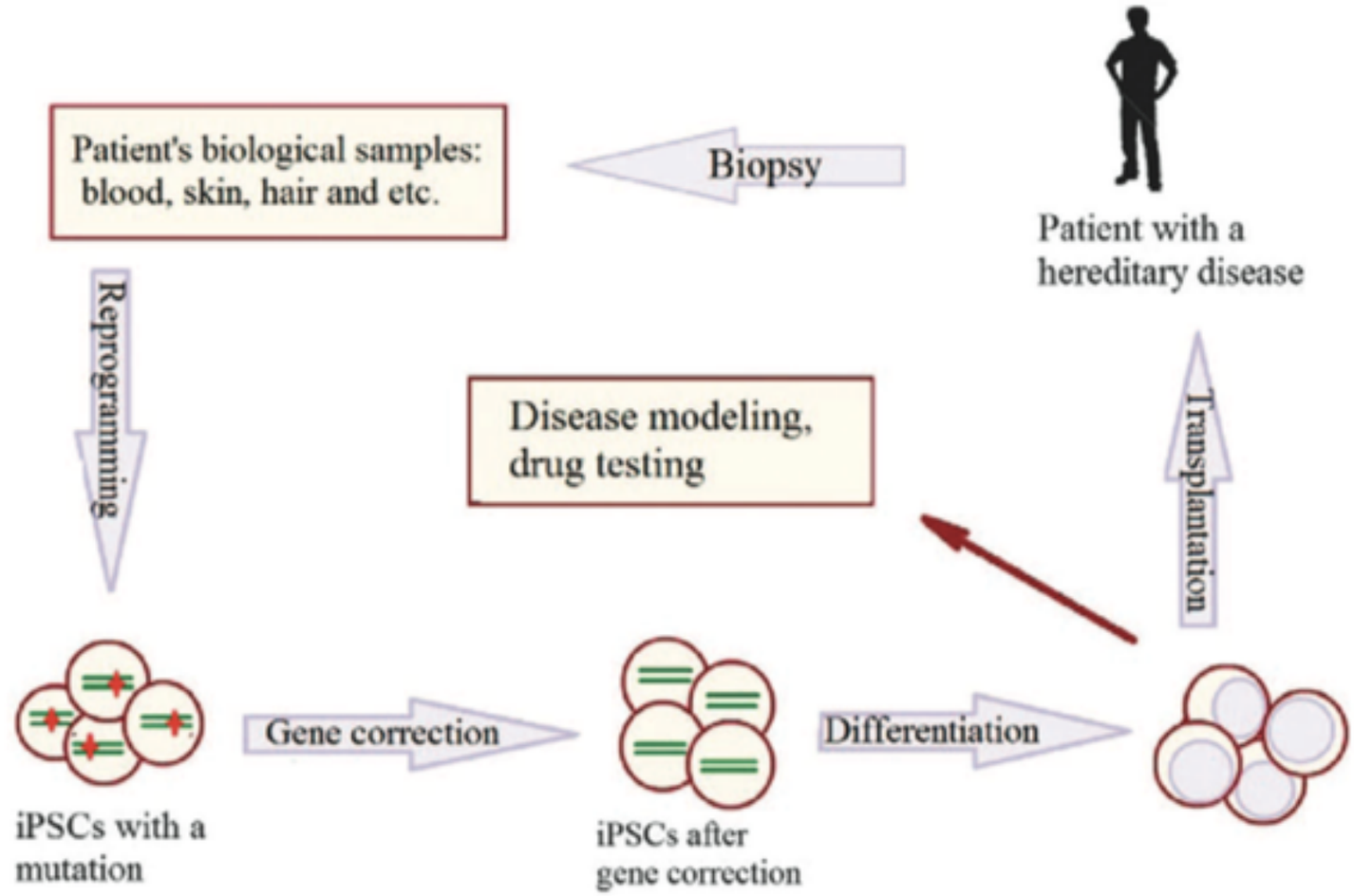


Figure 1 Application of genome editing in molecular medicine (gene therapy, disease modeling). iPCSs could be generated from somatic cells of the patient with monogenic diseases for correction, differentiation into cell types suitable for therapy and transplantation into a patient to restore the function

Genome-editing tools for stem cell biology

Modeling cancer with CRISPER-Cas9(10)

Table 2
Modeling cancer with CRISPR-Cas9.

Target genes	Types of mutation	Cell lines or cancer models	Malignancy	Reference(s)
TET2, RUNX1, DNMT3A, EZH2, NF1, SMC3	Loss of function (LOF) mutations	Lineage $^-$/Sca1$^+$/cKit$^+$ (LSK) cell	Myeloid malignancies	[27]
APC, P53, KRAS, SMAD4	Gain of function (GOF) of *Kras* and LOF of *APC, P53, SMAD4*	Human small intestinal stem cell	Colorectal cancer	[22]
APC, P53, KRAS, SMAD4	GOF of *KRAS* and LOF of *APC, P53, SMAD4*	Human intestinal stem cells	Colorectal cancer	[28]
PTEN, P53	Indels	Mouse 3T3 cells	Liver cancer	[30]
NKX2-1, PTEN, APC	Loss of function mutations	A $KRAS^{G12D}$-driven lung cancer mode	Lung cancer	[23]
MCL-1	Indels	Human Burkitt lymphoma (BL) cells	Lymphoma	[40]
CD74, ROS1	Interchromosomal translocation	HEK 293T cells; non-transformed immortalized lung epithelial cells (AALE)	Lung cancer	[34]
EML4, ALK	Intrachromosomal inversion within a single arm	HEK 293T cells	Lung cancer	[34]
KIF5B, RET	Intrachromosomal inversion across both arms	HEK 293T cells	Lung cancer	[34]
NPM, ALK	Interchromosomal translocation	HCT116 cell line	Lymphoma	[33]
mGeCKOa	LOF mutations	Non-small-cell lung cancer cell line	Lung cancer	[41]
KRAS, P53, LKB1	GOF of *KRAS* and LOF of *P53, LKB1*	Mouse dendritic cells	Lung cancer	[31]
EML4, ALK	Intrachromosomal inversion within a single arm	NIH/3T3 cells	Lung cancer	[35]
EML4, ALK	Intrachromosomal inversion within a single arm	Primary fibroblasts cells from mouse tail, or HEK 293T cells and ASB-XIV cell lines	Lung cancer	[36]
EWSR1, FLI1	Interchromosomal translocation	HEK293A cell line and human mesenchymal stem cells	Ewing sarcoma	[39]
RUNX1, ETO	Interchromosomal translocation	HEK293A cell line and CD34+ human hemopoietic/progenitor stem cells	Myeloid malignancies	[39]
PAX3-FOXO1	Chromosome translocation	Myoblast cells from fore and hind limb	Alevolar Rhabdomyosarcoma	[38]
H-RAS, CDKN2a	*H-RAS* expression plus knockdown of *CDKN2A*	Primary mouse embryonic fibroblast (*MEF*) cells; human melanoma A375 cells; C2C12 cells; NIH3T3 cells; LLc-1,B16-F10, MC-38 cell lines	Undifferentiated sarcoma with pleomorphic and rhabdoid	[32]
H-RAS, P53	*H-RAS* expression plus knockdown of *P53*	The same as the *"H-RAS, CDKN2A" line*	The same as the *"H-RAS, CDKN2A" line*	[32]
H-RAS, P53, PTEN	*H-RAS* expression plus knockdown of *P53* and *PTEN*	The same as the *"H-RAS, CDKN2A" line*	The same as the *"H-RAS, CDKN2A" line*	[32]

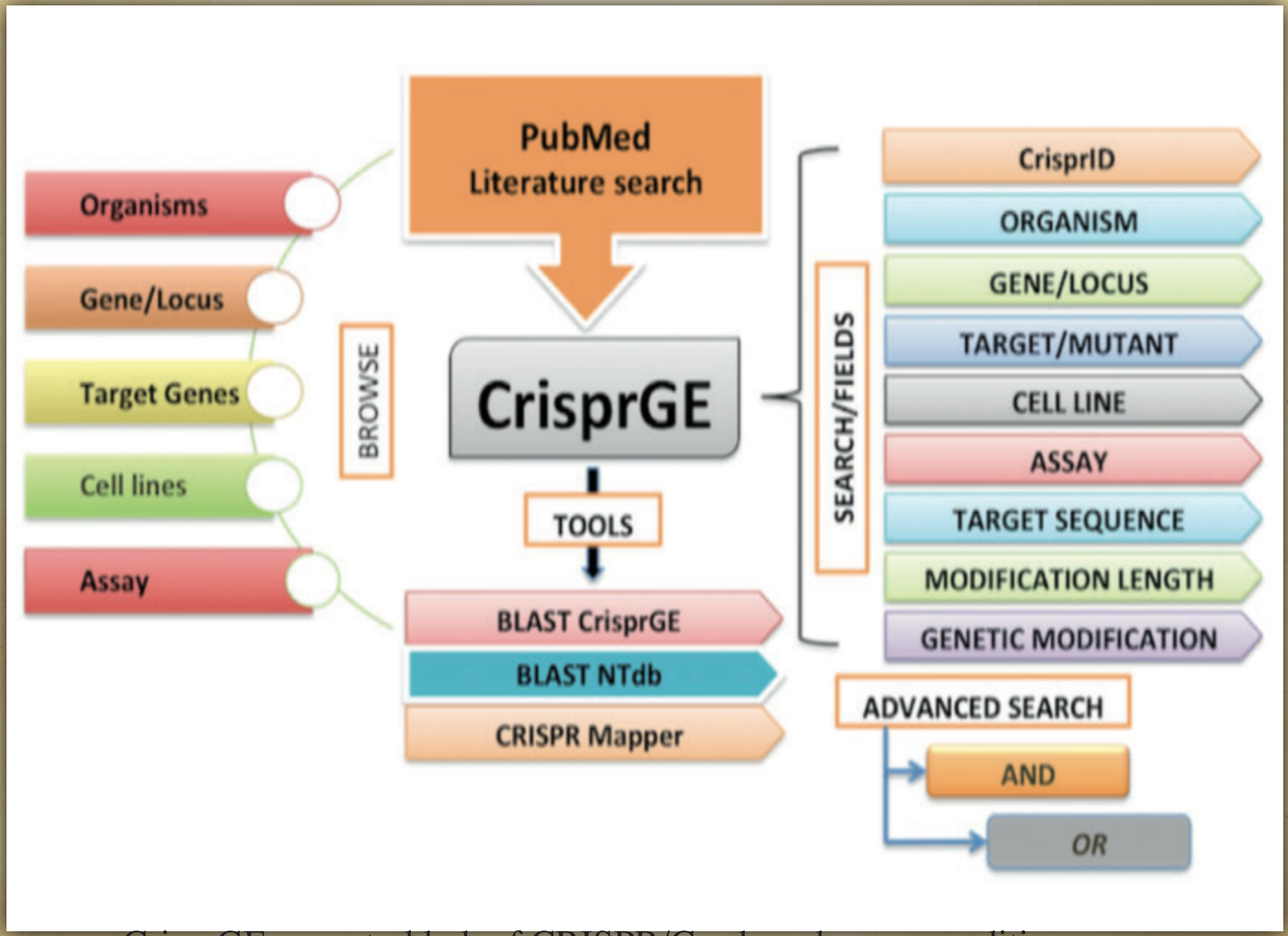
Development and potential applications of CRISPR-Cas9 genome editing technology in sarcoma

The **databases** to **design** CRISPR/Cas9(6)

Benchling: https://benchling.com/crispr
CasOT (CRISPR/Cas system (Cas9/gRNA) Off-Targeter): http://eendb.zfgenetics.org/casot/
CHOPCHOP: https://chopchop.rc.fas.harvard.edu/
COSMID: http://omictools.com/cosmid-s9890.html
CRISPR Design: http://crispr.mit.edu/
DESKGEN: https://www.deskgen.com/landing/#/
E-CRISP: http://www.e-crisp.org/E-CRISP/
sgRNA designer: http://www.broadinstitute.org/rnai/public/analysis-tools/sgrna-design
ZiFiT: http://zifit.partners.org/ZiFiT/

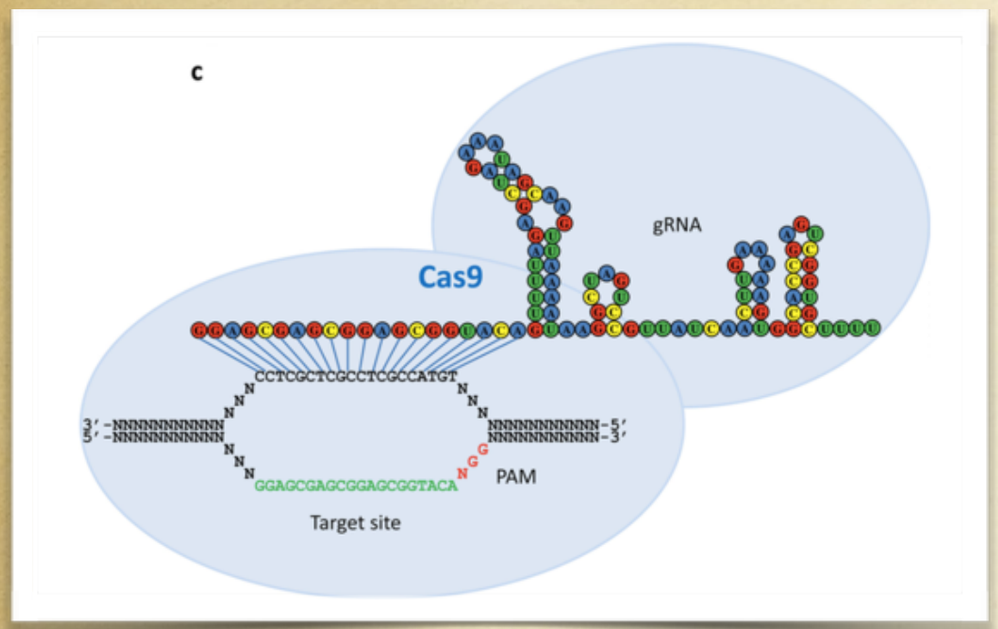
ALL LINKS ARE ACTIVE IN THE ONLINE PDF

CrisperGE(9)



CrisprGE: a central hub of CRISPR/Cas-based genome editing

Efficient In Vivo Genome Editing Using RNA-Guided Nuclease (7)



tia1l

Mutations in 17 out of 44 sequenced alleles

CCTGTGCTCTCCTGTTTTTAGGTATGTCGGGAACCTCTCCAGGGATGTTACGGAGGCCCT	Wild-type	
CCTGTGCTCTCCTGTTTTTAGGTATGTCGGGAACCTggggtatgtcgggaaTCCAGGGAT	+14	(-1,+15)
CCTGTGCTCTCCTGTTTTTAGGTATGTCGGGAACCagggatgttacGGATGTTACGGAGG	+4	(-7,+11)
CCTGTGCTCTCCTGTTTTTAGGTATGTCGGGAACataccaTCCAGGGATGTTACGGAGGC	+3	(-3,+6)
CCTGTGCTCTCCTGTTTTTAGGTATGTCGGGAACCTC--CAGGGATGTTACGGAGGCCCT	-2	[x5]
CCTGTGCTCTCCTGTTTTTAGGTATGTCGGGAACCTC---gGGGATGTTACGGAGGCCCT	-3	(-4,+1)
CCTGTGCTCTCCTGTTTTTAGGTATGTCGGGAACC----CAGGGATGTTACGGAGGCCCT	-4	
CCTGTGCTCTCCTGTTTTTAGGTATGTCGGGAACC-----AGGGATGTTACGGAGGCCCT	-5	
CCTGTGCTCTCCTGTTTTTAGGTATGTCGGGAAC------AGGGATGTTACGGAGGCCCT	-6	
CCTGTGCTCTCCTGTTTTTAGGTATGTC----------CAGGGATGTTACGGAGGCCCT	-11	[x3]
CCTGTGCTCTCCTGTTTTTAGGTATGTCGGGA----------TGTTACGGAGGCCCT	-13	
CCTGTGCTCTCCTGTTTTTAGGTATGTCGGGAACC----------CT	-23	

Figure 2.
Targeted indel mutations induced by engineered gRNA/Cas9 at the *tia1l* and *gsk3b* genes. For each gene, the wild-type sequence is shown at the top with the target sites highlighted in yellow and the PAM sequence highlighted as red underlined text. Deletions are shown as red dashes highlighted in grey and insertions as lower case letters highlighted in blue. The net change in length caused by each indel mutation is to the right of each sequence (+, insertion; −, deletion). Note that some alterations have both insertions and deletions of sequence and in these instances the alterations are enumerated in the parentheses. The number of times each mutant allele was isolated is shown in brackets.

Target gene	Site name	Sequence	No. of mismatches	Score	Gene	Editing
Tex15	Target	AGTTTCCACGTATATTGACTTGG	0	100	NM_031374.2	Yes
	OT-1	AGTTTCCA**T**GTAT**C**T**C**G**C**CTTGG	4	0.004	NM_030000	N.D.
	OT-2	AGTTTCCAC**T**TAT**G**T**CC**ACTCAG	4	0.013	NM_207206	N.D.
	OT-3	**G**GTTTCCACGT**T**TA**G**TG**G**CTGAG	4	0.042	NR_045436	N.D.
	OT-4	AGTTT**A**CA**A**GTA**G**ATT**C**ACTGAG	4	0.081	NM_178164	N.D.
	OT-5	AGTT**A**CCAT**G**A**G**TATTGACTGGG	4	0.231	NM_001165929	N.D.
	OT-6	**G**GTTTCC**TG**GTATATT**A**ACTGAG	4	0.379	NM_172606	N.D.
	OT-7	AGT**C**TCC**C**C**T**TATATTGACTAAG	3	2.343	None	N.D.
Cdk2	Target	AAGATTGGAGAGGGCACGTACGG	0	100	NM_016756	Yes
	OT-1	AAGATTGGA**A**AGGGC**T**C**T**T**T**TGG	4	0.019	NM_145465	N.D.
	OT-2	AAGAT**G**GGAGAGGGC**CT**GT**G**TGG	4	0.026	NM_019752	N.D.
	OT-3	**C**AGATTGGAGAGG**TG**ACGT**G**GAG	4	0.028	NM_010458	N.D.
	OT-4	AAGATTGGAGA**A**GGC**T**C**C**TATGG	3	0.042	NM_183294	N.D.
	OT-5	AAGAT**A**GGAGAG**A**GCA**G**GC**A**AGG	4	0.043	NM_001001178	N.D.
	OT-6	A**G**GATTGGAGAGGG**T**A**G**G**G**ACAG	4	0.053	NM_010833	N.D.
	OT-7	**G**AGAT**G**GGAGAGGGC**C**CGT**G**GGG	4	0.074	NM_001102563	N.D.
	OT-8	A**TT**ATTGGAGAGGG**G**AC**C**TATGG	4	0.081	NM_001109626	N.D.
	OT-9	AAGAT**C**GGAGAGGG**G**AC**C**TATGG	3	0.083	NR_004853	N.D.
	OT-10	**G**AGAT**A**GGAGAGGG**G**ACGT**G**GAG	4	0.094	NM_178371	N.D.
	OT-11	A**G**GATTG**A**AGA**A**GGCAC**C**TATGG	4	0.115	NM_007661	N.D.
	OT-12	**CG**GATTGG**C**GAGGGCAC**C**TATGG	4	0.155	NM_194444	N.D.
	OT-13	AAG**TG**TGT**A**GAGG**A**CACGTACAG	4	0.197	NM_001195730	N.D.
	OT-14	AAG**C**TT**TC**AGAGGGCA**T**GTATAG	4	0.217	NM_177386	N.D.
	OT-15	**C**AG**C**T**G**GGAGA**C**GGCACGTATGG	4	0.402	NM_008547	N.D.
	OT-16	**C**AG**G**T**A**GGA**A**AGGGCACGTAAAG	4	0.440	NM_178628	N.D.

schematic of U6 ABCB1 sgRNA_CMV Cas9 -GFP expression in **single plasmid** system (10)

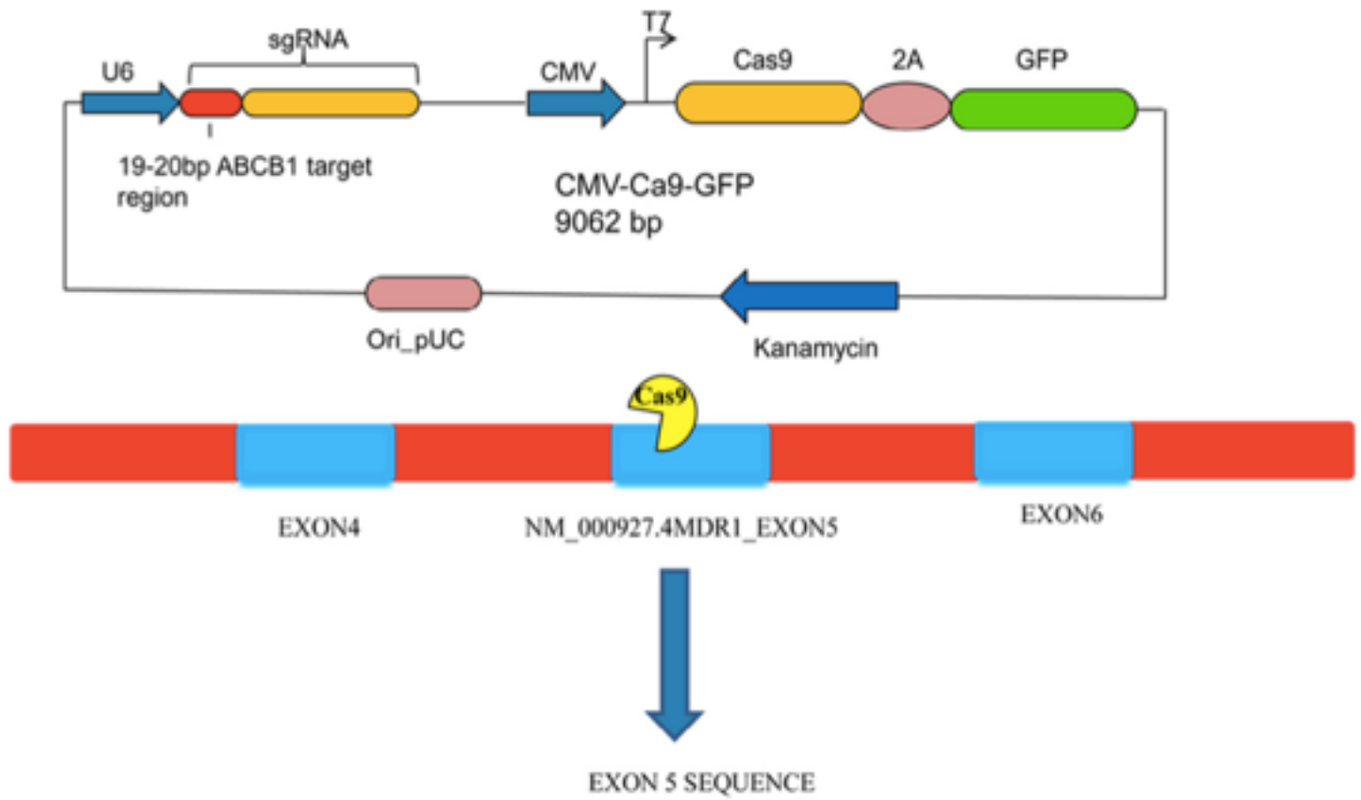


Fig. 3. Schematic of U6 ABCB1 sgRNA-CMV Cas9-GFP expression cassette in the single plasmid system. GFP is co-expressed from the same mRNA as the Cas9 protein via a 2A peptide linkage, which enables tracking of transfection efficiency. The exon of ABCB1 selected for guide RNA design is located on the 5th coding exon. The human U6 promoter is used to drive gRNA expression, while the CMV promoter drives expression of Cas9 and GFP proteins. The position of the frame shift that CRISPR-Cas9 knocks out is located at exon 5 within the ABCB1 gene (NM_000927.4MDR1). The red font is the 20 bp for gRNA, and AGG (yellow font) is the sequence of PAM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

strategy for generation of mouse PrP knock out clones on CRISPER/Cas9 -system (11)

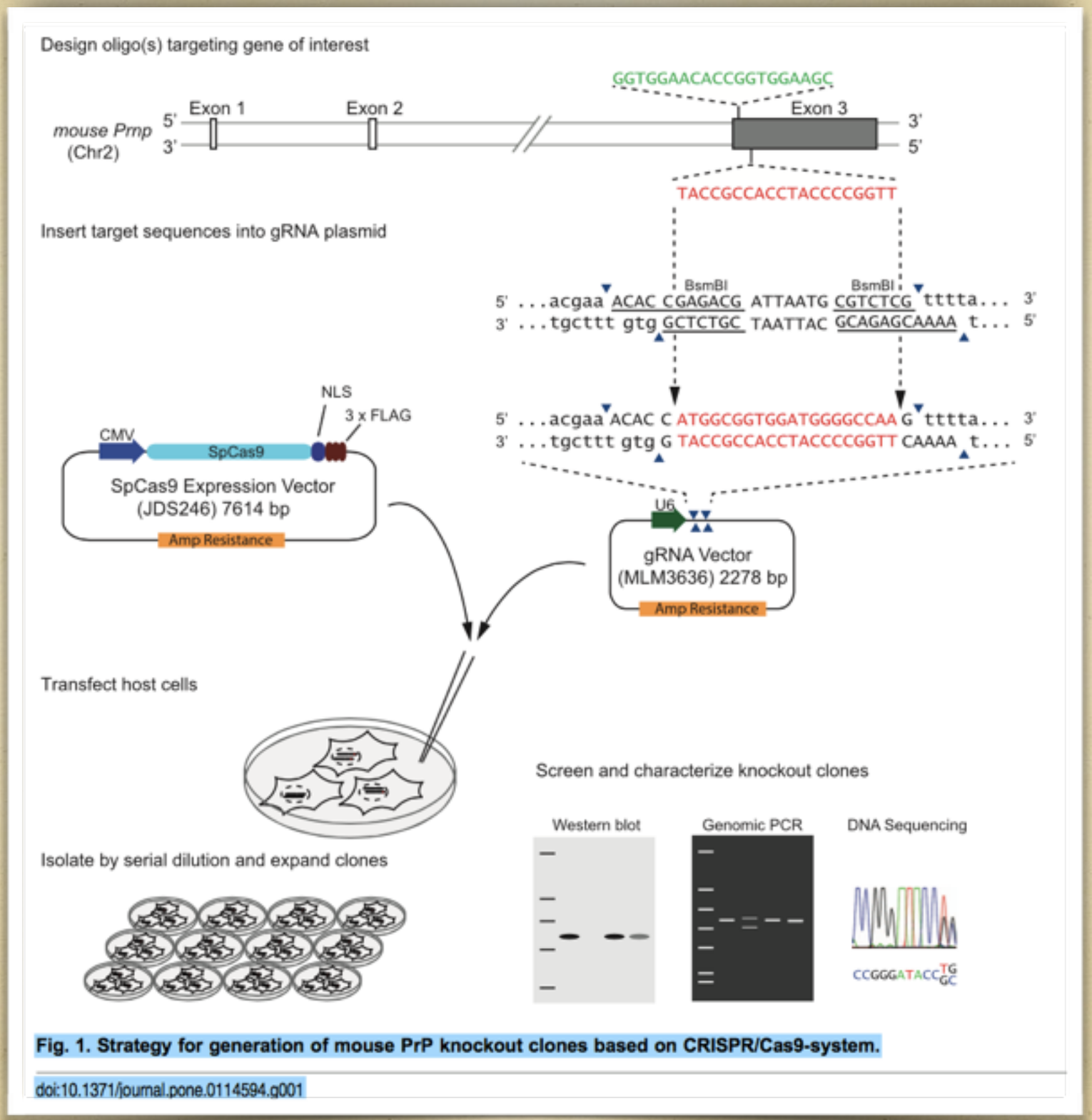


Fig. 1. Strategy for generation of mouse PrP knockout clones based on CRISPR/Cas9-system.

doi:10.1371/journal.pone.0114594.g001

CRISPR-Cas9-Based Knockout of the Prion Protein and Its Effect on the Proteome

Conclusions (5)

Applications of genome editing are still in their early stages.

Hopefully in the near future,

the application and replacement of particular regulation methods can also be successful and

in high-throughput manner, making the exploration of gene functions more precise and in-depth. Amazingly,

epigenetic regulation of genes by this technology will possibly open a new means in the field of functional epigenetic.

Conclusions

By taking advantages of these genome editing systems,

we are now able to extend functional mechanistic studies to more research fields.

Linked by technology,

molecular biologists and ecologists will now be able to better cooperate to explore the interesting and important issues,

such as

animal social behavior, and mechanisms of biodiversity maintenance.

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